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Genetic diversity and differentiation of the frankincense tree (*Boswellia papyrifera* (Del.) Hochst) across Ethiopia and implications for its conservation

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ABSTRACT

Boswellia papyrifera is used to produce frankincense, a bark resin that has been a commodity of domestic and international trade since ancient times. It is harvested from natural forests. The tropical dry forest (Terminalia–Combretum) woodland ecosystems in which *B. papyrifera* is one of the dominant species, are facing anthropogenic threats. In Ethiopia *B. papyrifera* populations have decreased tremendously to smaller and isolated remnant patches, and many forests in the North-western and North-eastern parts of Ethiopia completely lack recruitment of saplings. This regeneration bottleneck, in combination with adult mortality, threatens the persistence of the species. Devising an effective strategy to conserve wild genetic resources needs information on the genetic diversity and the pattern of genetic differentiation across the species area. In the present study we analysed adult trees sampled in twelve populations across the growing area of the species in Ethiopia for genetic diversity and spatial genetic differentiation using 10 polymorphic microsatellite loci. The mean level of observed and expected heterozygosity were 0.669 and 0.681 respectively, and these levels were similar for trees from larger populations and those from degraded populations. A moderate level of among populations genetic differentiation ($F_{ST} = 0.084$) was detected. Genetic distance between populations was correlated with geographic distance ($r = 0.663$, $p < 0.05$). STRUCTURE analysis distinguished four distinct genetic clusters corresponding to regions with different environmental conditions. In the Western populations we detected recruitment of many seedlings and saplings, which is a significant novel finding as most of the other populations are completely devoid of saplings. We conclude that currently a high level of genetic variation is still maintained in *B. papyrifera* adult trees across the species' range in Ethiopia including the highly degraded remnant *B. papyrifera* population patches scattered on farm and pasture lands. An effective conservation strategy for the species has to take into account the geographic distribution of source populations.

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1. Introduction

Widespread anthropogenic disturbance has resulted in the fragmentation and conversion of forest and woodland ecosystems into pasture and agricultural land (Lindenmayer and Fischer, 2006). Terminalia–Combretum woodlands in which *Boswellia papyrifera* is one of the dominant species (Vollesen, 1989) are no exception. These woodlands are threatened by various human-driven factors

in a large part of its geographical distribution (Ogbazghi et al., 2006; Abteu et al., 2012; Abiyu et al., 2010; Eshete et al., 2011). As a result *B. papyrifera* populations have decreased tremendously to ever smaller and isolated remnant patches. In addition, a severe regeneration bottleneck is threatening the persistence of this species as many populations are devoid of seedlings and small recruiting individuals. This situation has been described in Eritrea (Ogbazghi et al., 2006), Sudan (Abteu et al., 2012) and North-western Ethiopia (Abiyu et al., 2010; Eshete et al., 2011). *B. papyrifera* is used for the production of frankincense, the dried bark resin that has been a commodity of domestic and international trade since ancient times (Groom, 1981; Gebrehiwot et al., 2003). In modern days, *B. papyrifera* is an important economic resource

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because of the growing demand of frankincense for diverse industrial uses, including food flavours, cosmetic ingredients (e.g. lotions, soaps, and ointment formulation), and perfumes (Tucker, 1986; Coppen, 2005). Frankincense is exclusively harvested from trees in natural forests.

In Ethiopia *B. papyrifera* is commonly distributed in the dry lowlands in the Western, Northern, North-western, and North-eastern part of the country including the river basins of the Blue Nile, Jamma, Tekeze, and Zarema. The only population in the central part of the country is located along the Jamma River Gorge, which is a tributary to the Blue Nile river. Most of the populations lack rejuvenation. Tolera et al. (2013) indicated that no recruitment has occurred for the last 50 years in the North-western (Metema) populations. Some of the populations in the North-east are so severely degraded that only few scattered remnant trees are left on farmlands and inaccessible hill tops (Addisalem, personal observation, 2011). A high adult mortality is leading to ever smaller populations (Abiyu et al., 2010; Groenendijk et al., 2012). The ongoing threats were predicted to lead to a 90% decline in number of trees in 50 years and this loss might lead to a 50% associated loss of frankincense yield in 15 years (Groenendijk et al., 2012). Groenendijk et al. (2012) and Lemenih et al. (2014) underscored the need of implementing intensive management to sustainably conserve *B. papyrifera* and the incense production from the species.

Devising an effective strategy to conserve wild plant genetic resources requires basic information on the genetic diversity, differentiation among populations, and understanding the underlying processes that play role in determining the level and distribution of the genetic variation (Booy et al., 2000). Large populations of naturally outbreeding species usually have extensive genetic diversity (Frankham, 1995). In long-lived outcrossing plants such as trees, the majority of that genetic diversity occurs within populations. Pairs of populations farther away from each other will be genetically differentiated because of limited gene flow explained as movement of individuals, their seeds, or pollen over longer distances (Wright, 1943; Schaal et al., 1998). Geographic distance alone, however, may not explain all of the genetic differentiation because patterns of variation may also be impacted by environmental variables that are important for growth or survival of the species (Ledig and Fryer, 1972; Duminil et al., 2013). In addition, anthropogenic interference may affect the genetic variation and structure of a population (Frankham, 1995; Arens et al., 2007). Changes in population demography as a result of a rapid decline in population size may result in substantial loss of genetic diversity and may lead to increased differentiation across populations as a result of genetic drift if it continues for more than one or two generations (Hamrick et al., 1992; Frankham, 1995; Hamrick and Godt, 1996).

In this study we determined the genetic diversity and population structure of Frankincense tree (*B. papyrifera*) populations across the species range in Ethiopia. We hypothesized that (i) the genetic differentiation among populations is low since *B. papyrifera* is a long-lived and widespread species and (ii) populations farther away from each other are genetically more distant than populations close to each other due to the limited gene dispersal caused by spatial distance. We used the resulting information to identify to-be-conserved populations representative of the genetic diversity of the species.

2. Material and methods

2.1. Study species

B. papyrifera is one of the twenty species in the genus *Boswellia* (Burseraceae family; Vollesen, 1989). The species is a deciduous

monoecious tree with sweet scented flowers that are frequently visited by honeybees for pollen and nectar (Fichtl and Admasu, 1994). Fruits are about 2 cm long, usually containing three tapered seeds (Vollesen, 1989). Next to the stem being the source of frankincense, the flowers are the source of honey, the leaves provide animal fodder and various parts of the tree are used as traditional medicines.

2.2. Field sampling

Adult trees were sampled in twelve populations across the species range in Ethiopia (Supplementary .kmz file). Population were sampled from natural forests, or fragmented and degraded landscapes in areas where natural forests were not available. The geographical location and altitude of the populations were recorded using a handheld Garmin Dakota 20 GPS. Characteristics of the sampled populations (altitude, temperature, rainfall and soil) are listed in Table 1.

A total of 344 genotypes were sampled (25–43 individuals per population except for one population (AF-BIR) where sampling was possible only for 8 individuals because of inaccessibility of the area). As mechanisms of pollination and seed dispersal are not well known, a minimum of 25 m distance was kept between sampled individuals to reduce the risk of sampling genetically related individuals. Young leaves were sampled from growing shoots of the plants and dried and stored on silica gel until DNA extraction.

2.3. DNA extraction, PCR amplification and genotyping

Total DNA was isolated from dried young leaves following the cetyl trimethylammonium bromide (CTAB) protocol of Fulton et al. (1995) modified with 2% pvp-40 in the extraction buffer and 1% mercaptoethanol in the microprep buffer, added immediately before use, and followed by purification using DNeasy (Qiagen, Venlo, The Netherlands) according to Smulders et al. (2010). DNA yield and quality were visually assessed on a 1% agarose gel. Ten polymorphic microsatellite markers: Bp02, Bp11, Bp17, Bp18, Bp20, Bp21, Bp22, Bp23, Bp29 and Bp39 (Addisalem et al., 2015) were used for genotyping. PCR amplification was in a 10 µl reaction mix containing 4 µl 2 ng/µl DNA, 5 µl MP mix from Qiagen kit, 0.8 µl (2 µM) universal fluorescently labelled primer and 0.2 µl mix of the forward and reverse primers. For fluorescently labelling the forward primers were extended with a universal M13 sequence (AACAGGTATGACCATGA) at the 5' end (Schuelke, 2000) while the reverse primers were tailed with GTTT at their 5' end according to Brownstein et al. (1996) to reduce stutter bands. The PCR cycling profile consisted of 15 min denaturation at 95 °C, followed by 30 cycles of 30 s denaturation at 94 °C, 45 s annealing at 56 °C and 45 s extension at 72 °C. This was followed by 8 cycles at 53 °C annealing temperature to facilitate the annealing of the fluorescent dye-labeled M13 primer, and a final extension step of 10 min at 72 °C. After amplification 10 µl water was added for dilution and increase the volume of the product. Fluorescently labelled amplicons were resolved on a 4200 or 4300 Licor DNA analyzer. Bands were scored manually.

2.4. Data analyses

Based on the microsatellite data, the total number of alleles, effective number of alleles, mean values of expected and observed heterozygosity, and inbreeding coefficient were estimated and mean values over all loci and populations computed using GenAlEx 6.5 (Peakall and Smouse, 2006, 2012). The genetic differentiation based on allelic frequency (F_{ST}), AMOVA, and pair-wise Nei's unbiased genetic distance were also analysed using this program.

Table 1

Ecological description of the sampled regions according to Eshete et al. (2011), Teshome (2013), Alemu et al. (2015) and own data/observations. NA = data not available; KTD: Kola-Temben-Dambar; AB: Abergelle; TRG: Tekeze-River-Gorge; AF-BIR: Afar-Bir Hale; B-KUR: Benishangul-Kurmuk; B-SHK: Benishangul-Sherkole; MAW: Metema-Awlaba; MQ: Metema-Quara; JRG: Jamma-River-Gorge; KHB: Kafa-Humera-Baeker; KHW: Kafa-Humera-Waldaba; TA-SJ: Tach-Armachew-Sanja. The geographic coordinates are in decimal form.

Pop.	Latitude	Longitude	Altitude (m)	Rainfall (mm)	Maximum temp. (°C)	Population size (ha)	Rejuvenation	Density (stems/ha)	Soil type
KTD	13.812969	38.995745	1771	700	NA	<10	Absent	<50	NA
AB	13.457950	38.858440	1622	849	29.3	>50	Absent	200–400	Clayey, shallow, not fertile
TRG	13.744338	38.207686	1015	857	NA	20–50	Absent	50–100	NA
AF-BIR	13.892760	40.092710	597	301	45	<10	Few seedlings	<50	NA
B-KUR	10.563689	34.303058	680	960	39	>50	Abundant seedlings and saplings	>400	Clayey to sandy-loam, red, deep, moist, fertile
B-SHK	10.660723	34.692611	879	1245–1350	42.0	>50	Abundant seedlings and saplings	100–200	Clayey to sandy-loam, red, deep, moist, fertile
MAW	12.690783	36.313999	882	800–900	35.7	>50	Absent	200–400	Clayey, deep, fertile
MQ	12.566644	36.067952	597	800–900	35.7	>50	Absent	200–400	NA
JRG	10.065459	38.467988	1120	880	NA	10–20	Absent	50–100	NA
KHB	13.959036	36.858947	811	400–650	41.7	>50	Few seedlings present	200–400	Combisols, luvisols, vertisols
KHW	14.055500	37.129460	985	400–650	41.7	20–50	Few seedlings present	200–400	Combisols, luvisols, vertisols
TA-SJ	13.093871	37.268437	953	750	NA	20–50	Absent	100–200	NA

The correlation of genetic distance and geographic distances of populations were analysed using Isolation by distance, web service (IBDWS; Jensen et al., 2005) using pairwise genetic distances calculated using GenAlEx 6.5 and geographical distances calculated with Geographic Distance Matrix Generator 1.2.3 (Ersts, [internet]). The coefficient of determination was calculated in order to determine the proportion of the genetic distance attributable to geographic distance.

A Principal Component Analysis (PCA) was performed to explore the genetic relationship of the populations using GenAlEx 6.5. Individual-based population assignment was used to identify differentiated genetic clusters using the Bayesian assignment clustering method implemented in STRUCTURE 2.3.4 (Pritchard et al., 2000). The optimal number of genetic clusters (K) was determined using a 10,000 cycle burn-in period and 100,000 Monte-Carlo Markov Chains (MCMC), using the admixture model (which assumes individuals may have mixed ancestry) and assuming correlated allele frequencies among subpopulations. Simulations were repeated 20 times for $K = 1$ to $K = 10$. The K that best explained the data (most likely number of clusters) was inferred following Evanno et al. (2005). STRUCTURE analysis calculated membership coefficient of individuals (individuals Q-matrix) for each of the defined genetic clusters and the proportion of ancestry of each population in each of the clusters (population Q-matrix) by averaging the membership coefficient of all the individuals of a population. Subsequently, populations were assigned to a cluster based on the highest average proportion of ancestry, no matter what this value was for the rest of the clusters (Porrás-Hurtado et al., 2013).

3. Results

3.1. Sampling across Ethiopia

Most populations visited for sampling were degraded, lacking rejuvenation and young individuals. The most depauperate populations were AF-BIR and KTD in the North eastern region, where few trees were scattered across hilltops and farmland. In contrast, we discovered populations in the West that were well stocked with seedlings and young recruiting individuals (saplings). This discovery is new for Ethiopia, as several authors (Eshete et al., 2011; Groenendijk et al., 2012; Tolera et al., 2013) reported the complete absence of recruitment for at least five decades in the North western and North eastern parts of Ethiopia. The West is less affected by agricultural activities and the woodlands are relatively intact.

Table 2

Performance of the microsatellite markers on 344 samples taken across Ethiopia. Total number of alleles scored per locus (A_L), mean values of observed (H_O) and expected heterozygosity (H_E), and inbreeding coefficient (F_{IS}) per locus across 10 populations (excluding AF-BIR and JRG) of *B. papyrifera* in Ethiopia.

Locus name	A_L	H_O	H_E	F_{ST}	F_{IS}
Bp02	10	0.713	0.735	0.061	0.031
Bp11	11	0.311	0.310	0.061	−0.002
Bp17	11	0.586	0.730	0.096	0.197
Bp18	10	0.526	0.527	0.121	0.002
Bp20	12	0.806	0.791	0.077	−0.019
Bp21	24	0.829	0.815	0.084	−0.017
Bp22	28	0.795	0.742	0.065	0.049
Bp23	24	0.820	0.790	0.093	−0.038
Bp29	14	0.622	0.554	0.100	−0.123
Bp39	22	0.773	0.811	0.081	0.047
Mean	16.6	0.669	0.681	0.084	0.013

3.2. Genetic diversity detected with the microsatellite markers

Ten of the 12 populations were genotyped with the 10 SSR loci. Populations AF-BIR and JRG were genotyped with only 5 loci because of failure in amplification and weak signals in the other loci, probably due to poor DNA quality as only mature leaves could be sampled. An average of 16.6 different alleles were scored across the 10 microsatellite loci used, ranging from 10 alleles at Bp02 and Bp18 to 28 alleles at Bp22 (Table 2). As expected, this was much higher than the number of alleles detected when developing the markers (6–12 alleles per marker, average 8.3), as these were tested on a small set of samples only (Addisalem et al., 2015). The observed heterozygosity (H_O ; average 0.669) was similar to the expected heterozygosity (H_E ; average 0.681) across the markers, and hence the inbreeding coefficient (F_{IS}) was very low (average 0.01, range −0.034 to 0.077). Only marker Bp11 had a lower level of heterozygosity (0.31), even though it amplified 11 different alleles.

3.3. Intra-population genetic diversity

The heterozygosity for each population varied between 0.622 to 0.727 for H_O and 0.661 to 0.725 for H_E (Table 3). The heterozygosity of KTD, which is a population of scattered remnant trees on farmlands, was comparable ($H_O = 0.701$ and $H_E = 0.661$) to that of the large populations. The mean number of alleles (N_A) and effective number of alleles (N_E) per populations were 7.78 (range 3.6–8.9)

Table 3

Allele frequencies and heterozygosity, averaged across 10 loci, for 12 populations of *B. papyrifera* in Ethiopia. *N*: number of individuals genotyped per population; *N_A*: total number of alleles; *N_E*: effective number of alleles; *H_O*: observed heterozygosity; *H_E*: expected heterozygosity.

Population	<i>N</i>	<i>N_A</i>	<i>N_E</i>	<i>H_O</i>	<i>H_E</i>	<i>F_{IS}</i>
TRG	30	6.9	4.2	0.679	0.692	0.024
AB	30	6.7	3.9	0.635	0.676	0.049
KTD	30	5.9	3.3	0.701	0.661	0.061
AF-BIR ^a	8	3.6	2.5	0.810	0.590	−0.436
B-KUR	28	8.6	4.1	0.669	0.666	−0.018
B-SHK	43	8.7	4.3	0.637	0.671	0.028
MQ	30	8.1	3.8	0.669	0.668	−0.004
MAW	30	8.7	4.4	0.727	0.725	−0.015
JRG ^a	25	5.4	3.1	0.660	0.640	−0.081
TA-SJ	30	7.7	3.9	0.698	0.683	−0.034
KHB	30	7.6	3.8	0.622	0.669	0.077
KHW	30	8.9	4.4	0.651	0.693	0.057
Mean		7.78	4.02	0.669	0.680	0.010

^a Genotyped over 5 loci namely Bp02, Bp17, Bp18, Bp21 and Bp39.

and 4.02 (range 2.5–4.4), respectively (Table 3), and also *N_E* was similar across populations.

3.4. Inter-population differentiation and genetic relationship

The average *F_{ST}* across all 12 populations, based on 5 markers, was 0.0109 ($P < 0.001$; Table 3). When the two populations (JRG and AF-BIR) that were genotyped with only 5 markers, were excluded from the calculation, the *F_{ST}* for 10 populations based on all 10 markers was slightly lower (0.084; $P < 0.001$). Nei's unbiased pair-wise genetic distances ranged from 0.017 to 0.411 (Table 4). Generally, a large genetic distance was observed between the Western (B-SHK and B-KUR) and North eastern region (KTD, AB, and TRG) populations, which are geographically most distant. B-SHK and AB were genetically the most distant from each other (0.411) followed by B-SHK and TRG (0.386), and B-SHK and KTD (0.364).

Genetic distance was significantly correlated ($r = 0.663$, $p < 0.001$) with geographic distance (Fig. 1). About 44% ($r^2 = 0.44$) of the genetic distance could be due to the geographic distance among the populations.

3.5. Population structure

Consistent with the low *F_{ST}* and the fact that most diversity is present among sampled trees within populations, a PCA plot revealed somewhat differentiated populations, with a large overlap of individuals from different populations (Fig. 2).

With regard to the analysis of population structure, the most likely number of clusters (*K*) was 4 (not shown). The STRUCTURE analysis identified two clusters containing 3 populations and two clusters each with two populations (Table 5). Two of the four

Fig. 1. Correlation of genetic distance (*F_{ST}*) with geographic distance (Km) for all possible pairs of the 10 surveyed populations of *B. papyrifera* in Ethiopia.

clusters were clearly distinct while the other two showed individuals with mixed posterior probability of cluster membership. One of the populations (TA-SJ) was assigned to the cluster with low (45.9%) posterior probability (Table 5). These four genetic clusters corresponded to different geographical regions but also to different environmental conditions. The clusters are hereafter referred to as North Eastern (NE), Western (W), North Western (NW) and Northern (N) clusters based on the geographical locations of the accessions (Fig. 3). The analyses placed TRG, AB, and KTD within one cluster (NE cluster). The maximum geographic distance among these population was 85 km. B-KUR and B-SHK were grouped together as one cluster (W cluster) and these populations were 44 km apart. MQ and MAW corresponded to a single cluster (NW cluster). MQ and MAW were only 30 km apart. TA-SJ and the populations from the Northern part of the country (KHB, KHW) were assigned together (N cluster). Although TA-SJ is located more than 100 km from KHB and KHW, it was assigned to this cluster but with a posterior probability below 0.5 (0.459), and the probability for the next cluster, NW, was 0.339.

4. Discussion

4.1. Intra-population genetic diversity of *B. papyrifera* in Ethiopia

The present study examined the genetic diversity and pattern of population differentiation in the Frankincense tree *B. papyrifera* in Ethiopia. The populations of *B. papyrifera* have declined tremendously in size, are fragmented and degraded, and many

Table 4

Nei's unbiased pairwise genetic distance (below diagonal) and geographic distance (in km, above diagonal) among sampled *B. papyrifera* populations in Ethiopia.

	TRG	AB	KTD	B-KUR	B-SHK	MQ	MAW	TA-SJ	KHB	KHW
TRG	0	77	86	553	514	266	236	125	148	122
AB	0.163	0	42	591	550	319	289	177	223	198
KTD	0.178	0.145	0	626	585	346	316	203	231	203
B-KUR	0.183	0.328	0.258	0	44	295	323	429	469	496
B-SHK	0.386	0.411	0.364	0.207	0	260	287	390	436	462
MQ	0.294	0.338	0.287	0.259	0.241	0	30	143	177	202
MAW	0.194	0.242	0.214	0.236	0.248	0.066	0	113	153	176
TA-SJ	0.206	0.209	0.149	0.175	0.243	0.152	0.153	0	106	108
KHB	0.179	0.163	0.094	0.231	0.261	0.210	0.127	0.076	0	31
KHW	0.196	0.183	0.140	0.230	0.227	0.189	0.109	0.083	0.017	0

Fig. 2. Principal Component Analysis (PCA) of the 311 *B. papyrifera* individuals from 10 populations based on genotyping with 10 microsatellite loci. The first and second axis explained 6.38% and 5.08% of the genetic variation. For population abbreviations see Table 1.

Table 5

Proportion of ancestry (calculated as average membership coefficient of all the individuals of a population) in each of the four *B. papyrifera* genetic clusters. Each colour represents one genetic cluster (yellow = NE, Green = W, Blue = NW, and red = N).

Populations	Average proportion of ancestry in each of the four clusters				Genetic clusters in which populations assigned
1 TRG	0.776	0.026	0.158	0.041	NE
2 AB	0.823	0.015	0.100	0.062	
3 KTD	0.852	0.018	0.077	0.052	
4 B-KUR	0.134	0.590	0.222	0.054	W
5 B-SHK	0.026	0.875	0.060	0.038	
6 MQ	0.043	0.047	0.821	0.090	NW
7 MAW	0.057	0.072	0.617	0.253	
8 TA-SJ	0.142	0.060	0.339	0.459	N
9 KHB	0.125	0.018	0.066	0.790	
10 KHW	0.089	0.043	0.104	0.764	

populations in the North and Northwest of Ethiopia have no recruitment of young saplings at all (Eshete et al., 2011; Tolera et al., 2013).

A small population size and decreased connectivity (gene flow) may erode the populations' genetic variability and drive strong spatial genetic structuring (Schaal et al., 1998; Kramer et al., 2008). In this study, however, we detected ample genetic variation ($N_A = 8$) and no inbreeding ($H_O = 0.669$, $H_E = 0.681$, $F_{IS} = 0.01$) in the adult trees of the populations we sampled from various parts of Ethiopia, including those from the most degraded populations. The heterozygosity was comparable to estimates obtained for other tropical tree species for example *Acacia senegal* (mean $H_O = 0.709$, Omondi et al., 2010), one of the Terminalia–Combretum woodland tree species co-occurring with *B. papyrifera* in Ethiopia. One of the *B. papyrifera* populations (KTD) consisted of only remnant trees scattered on farmlands, yet it had the second highest heterozygote in the study ($H_O = 0.701$). In *B. papyrifera* the intraspecific genetic diversity apparently is still present in the mature trees. This is probably due to the long life span of individual trees (Smulders et al., 2008) in combination with the fact that the ongoing forest fragmentation and degradation processes in Ethiopia are relatively recent events. We do not know whether

B. papyrifera tolerates selfing, but the main threat to the populations is one of recruitment (Eshete et al., 2011; Tolera et al., 2013).

Forests in Ethiopia were still unaffected by man in 1900 (EFAP, 1994; Reusing, 2000). Lemenih et al. (2007) related the degradation of these dry tropical forest ecosystems to continuous human settlement, which was intensified since the late 1960s. Tolera et al. (2013) studied tree rings and estimated that the mature trees in existing *B. papyrifera* forests established themselves from seeds between 1903 and 1955, but not later. Their results implied an absence of regeneration during the last 60 years. Based on our population genetic data the *B. papyrifera* trees that we sampled still represent the diversity in their parents, which grew in the large and continuous forests that existed in the 19th century.

A second positive note is that we discovered populations in the Western region (Benishangul-Gumuz) with a high density of seedlings and saplings, indicating that natural regeneration still takes place in these populations. This region has fewer agricultural activities and the forests are relatively undisturbed. Traditionally this low and hot region did not see cattle herding because of the occurrence of Trypanosoma. This situation is currently changing but the livestock population in the region of Assosa is still very low, and particularly in Boswellia woodlands no cattle occur, only a few

Fig. 3. Geographic distribution of the four genetic clusters of *B. papyrifera* in Ethiopia. Clusters are identified based on STRUCTURE analysis. Each arrow indicates one population in a cluster. The cluster is subdivided into populations with the black solid line. AF-BIR and JRG were not included in the analysis. For population abbreviations see Table 1.

goats, donkeys and camels (Addisalem, personal observation). Possibly the deeper soil and the higher amount of rainfall also contribute to good microsites for seedling development. This region has a much higher amount of rainfall (as much as 1350 mm annual precipitation in B-SHK) than the North of Ethiopia (Table 1; Eshete et al., 2011; Teshome, 2013; Alemu et al., 2015).

4.2. Population differentiation

The average F_{ST} across all the 12 populations, based on data from 5 markers, was 0.0109 ($P < 0.001$) while it was a bit lower (0.084) when the two populations (AF-BIR and JRG) were excluded from the calculation. The higher F_{ST} among all 12 populations may be caused by geographical distance, isolation of the populations and/or adaptation to the environment. JRG is geographically the most distant while AF-BIR is isolated from other populations by mountains. The latter is also located in very harsh climatic conditions, namely a very hot ($>45^{\circ}\text{C}$) area with rainfall as low as 300 mm. These populations would be interesting for a follow-up study. Sampling of young leaves, as we did here, turned out to be problematic as rainfall was irregular and unpredictable. A possible alternative would be to sample bark tissue for DNA extraction (e.g., Novaes et al., 2009), but this needs to be tested for *B. papyrifera* as the exudate in the bark may hamper the extraction of good quality DNA.

The F_{ST} obtained was comparable to the genetic differentiation observed in the out-crossing tree species *Swietenia macrophylla*

($F_{ST} = 0.097$, Lemes et al., 2003) and in *Populus euphratica* ($F_{ST} = 0.093$, Wang et al., 2011), a dioecious, out-crossing and wind-pollinated desert tree species. The sampled *B. papyrifera* populations were at least 30 km apart (MAW and MQ in the North west). *B. papyrifera* is a species characterized by bright and fragrant flowers (Fichtl and Admasu, 1994). The flowers are frequently visited by honeybees for pollen and nectar (Fichtl and Admasu, 1994), hence honeybees are considered one of the pollinators of the species. Insect pollination (entomophily) was confirmed in two closely related species, *Boswellia serrata* (Sunnichan et al., 2005) and *B. ovalifoliolata* (Raju et al., 2012), while wind pollination was ruled out in *B. serrata*. The seed dispersal mechanism of the species is not well known. In *B. ovalifoliolata*, a closely related species, the seeds disseminate up to a distance of 400 m (Raju et al., 2012) indicating limited seed dispersal. For *B. papyrifera* such a seed dispersal distance may not be very effective to genetically connect populations. Hence, the isolation-by-distance pattern of genetic differentiation across populations likely is the consequence of pollen dispersal by insects.

In its growing regions the species is also distributed along river basin networks, with occasional chances for long distance seed dispersal along the traversing rivers. Likewise, *Populus nigra*, a riparian species in Europe, had a F_{ST} of 0.081 (based on microsatellites) among river systems (Smulders et al., 2008). However, the contribution of water to seed dispersal was lower than anticipated in many riparian species. We do not know whether water is an effective seed dispersal agent for *B. papyrifera*.

There was a clear pattern of isolation-by-distance (IBD), which explains 44% of the variation among populations. The findings support our prediction that *B. papyrifera* populations are spatially structured as the result of their geographical isolation. However, genetic differentiation may also be caused by genetic drift and natural selection (Ledig and Fryer, 1972; Slatkin, 1987; Duminil et al., 2013). The individual-based STRUCTURE analysis resolved the 10 populations into four genetically differentiated clusters that correlated with the geographical locations of the sampled populations. The four clusters, North eastern (NE), Western (W), North western (NW) and Northern (N), also correspond to environmentally different conditions in terms of precipitation and soil conditions. The NE populations grow in regions characterized by higher altitude (above 1000 m a.s.l.), modest levels of rainfall (mean annual precipitation 850 mm), cooler temperatures (average maximum temperature 29.3 °C) and less fertile, clayey and shallow (15 cm deep) soil (Eshete et al., 2011). The W populations are located at lower altitude (below 900 m a.s.l.) in hot (maximum temperature of 39–42 °C) but moist areas that receive up to 1350 mm annual precipitation. The soil is deep red, and clayey to sandy-loam in texture (Teshome, 2013). The NW populations are located at lowland altitudes (below 900 m a.s.l.), which are also hot (around 36 °C) and receive modest precipitation (800–900 mm) annual precipitation. The soil is deeper (27 cm), more fertile and clayey in texture (Eshete et al., 2011). Finally, the N populations are located in areas that are dry (less than 650 mm precipitation) and hot (maximum temperature 41 °C) (Alemu et al., 2015). Hence, the population genetic differentiation detected in *B. papyrifera* may differentiate populations that are also adapted to local environmental conditions, caused by precipitation, temperature, altitude and soil conditions or combinations of these factors. This needs to be confirmed with further studies such as common garden experiments or provenance studies.

4.3. Conservation of populations *in situ* and *ex situ*

High levels of genetic variation are still present in the adult trees in *B. papyrifera* populations across the species' range in Ethiopia. Yet, the demographic threats should not be discounted because most of the extant populations of *B. papyrifera* are now degraded in terms of their size, spatially fragmented, and they lack recruitment of new generations of trees (Eshete et al., 2011; Groenendijk et al., 2012; Tolera et al., 2013).

Sampling for conservation and *in situ* management of genetic resources should ensure the majority of extant variation is preserved. Conserving among-population diversity should focus on reserving the most genetically distinctive populations whilst conserving within-population diversity should preserve large core populations that will not lose diversity due to genetic drift (Namkoong, 1988). In *B. papyrifera*, therefore, selection of populations for conservation should take into account the geographical distance among the populations, but also the existence of distinct clusters, (NE, W, NW and N) and the fact that they grow in different environmental conditions. Within each cluster, populations of conservation priority have to be selected taking into account the level of genetic diversity, the regeneration status of the populations and the level of threat populations are experiencing. Delineating large reserves with more than one population within each cluster would ensure a sample of a gene pool that captures the diversity and uniqueness existing in the standing populations of the species.

Our findings suggest that the conservation value of degraded adult *B. papyrifera* populations under human influenced landscapes should not be overlooked, because such populations (e.g. KTD) still maintain genetic diversity comparable to the genetic diversity of the continuous forests that once existed. However, the *in situ* or *circa situm* (conservation in human influenced landscapes) man-

agement of such depleted populations needs active enrichment planting, not only within the current populations to replace dying old trees, but also in areas between the current populations, to improve and facilitate the genetic connectedness of individuals and populations, and thereby to ensure that the genetic diversity is maintained to the future.

Although only scarce genetic data were obtained so far, the population at Afar (AF-BIR), because of its geographical isolation, the depletion of the populations, and its ability to grow in the harsh environmental conditions (Table 1) may deserve special attention of conservation measures. This population is marginal in terms of microclimate (located in the hottest place on earth, the Danakil depression in Ethiopia, which barely supports vegetation). The effects of climate change in the region, namely a rise in temperature and declining and unpredictable rainfall (Joanne et al., 2005; NMA, 2007; EPA, 2010), on the population dynamics (e.g. regeneration, population expansion and shift in ecological range) of the species is not known. However, it is expected that such trends of climate change may result in expansion of drought in arid areas such as Afar-Bir Hale, suggesting increasing risk of population degradation and subsequent risk of local extinction. In view of this, it is highly advisable to preserve this population *ex situ* in laboratory and field gene banks, additionally to more common *in situ* conservation. The same may account for the quite isolated JRG population.

The close correlation of the genetic clustering to very diverse environmental conditions suggests the need of establishing provenance trial plantations in each of the four sites, both to preserve all available local and regional genetic variation *ex situ*, and to use these as source populations for future breeding programs. Urgent action is needed if people keep valuing frankincense as an important commodity, both for local use as for the international market.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.foreco.2015.10.038>. These data include Google maps of the most important areas described in this article.

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